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Histochemical Analyses of the Fluid and the Solid State of the Adhesive Materials Produced by the Pre- and Postmetamorphosed Cyprids of *Balanus eburneus* Gould

(Figures 1-6; Tables 1-10)

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Two distinct eosinophilic zones of granules found in the larval (cyprid) cement gland of *Balanus eburneus* Gould are referred to as medial and inner granules, according to their morphological position within the gland. No such differentiation of the secretory materials are present in the adult cement cells. Histochemical tests show that the granules, which represent the fluid state of the cement material, in the cyprid and adult, are basic protein, characterized as collagenous substances. The *hardened* cement produced by the cyprid and the adult that is found on the basal plate are histochemically unreactive protein masses, but the two substances are not histochemically identical.

Introduction

In recent years, studies on barnacles have been concerned with the cement, its synthesis, and the cement-producing complex. Thus, the general morphology of the cement gland in the cyprid stage was reported by Bernard and Lane (1962), the histology by Walley (1969), and the ultrastructure of the gland and the attachment pads of the antennules by Nott (1970), Nott and Foster (1970), and more recently by Walker (1971).

The histology of the cement apparatus of several species of adult barnacles was described in detail by Lacombe (1966, 1967, 1970), Lacombe and Liguori (1969), and at the ultrastructural level, by Walker (1970).

Costlow (1959) demonstrated the presence of carbonic anhydrase in the shell-forming tissue of the adult barnacle. Arvy and Lacombe (1968), Arvy, Lacombe, and Shimony (1968), and Shimony and Nigrelli (1971a, b) reported the presence of succinic dehydrogenase, alkaline phosphotase, arylsulphatase, and polyphenoloxidase, respectively, in the cement and cement apparatus of the adult barnacle, while Walker (1971) demonstrated polyphenoloxidase in both the gland and in the secreted cement of the cyprid stage of *Balanus balanoides.*

On the basis of histochemical techniques, various opinions were presented as to the chemical nature of the barnacle cement. Hillman and Nace (1970) and Shimoy (1971) concluded that the cement laid down by the attached cyprids was collagen, an opinion with which we concur in this report. Walker (1970, 1971) and Saroyan, *et al* (1970a) stated that the cement substances of the cyprid and the adult were proteins with phenolic groups, while Lacombe (1968) concluded that the cement in the adult barnacle consisted of acid mucopolysaccharides.

These reports show the confusion in defining the chemical nature of the cement at a particular stage. The present paper deals with histochemical analyses of the fluid and solid states of the adhesive materials produced by the pre- and post-metamorphosed cyprids of *Balanus eburneus* Gould.

Materials and Methods

Adult barnacles were collected locally or obtained from Biscayne Bay (Florida), attached to aluminum plates, and air shipped the same day to New York. The cyprids were raised in the laboratory and allowed to metamorphose to the barnacle stage in beakers. The glass around each barnacle was then cut in such a way that it served as a slide for microscopical examinations following treatment for various histochemical reactions.

Cyprids and barnacles were fixed in 10% buffered formalin, Bouin's fluid, Heidenhain's Susa, Zenker's, and Carnoy's solutions for general histological and histochemical observations. Where necessary, 5% nitric acid was used for

[1] Supported by ONR Contract N-00014-68-C-0334.

decalcification. After fixation and decalcification the animals were embedded in paraplast and sectioned at 6 μ. The various histochemical methods employed in this study are summarized in Tables 1-5.

TABLE 1. GENERAL HISTOLOGICAL AND HISTOCHEMICAL METHODS USED TO DEMONSTRATE VARIOUS COMPONENTS OF THE CEMENT APPARATUS OF THE BARNACLE, *Balanus eburneus* GOULD

Methods	Fixatives	References	Purposes
Hematoxylin and eosin	10% neutral formalin	Thompson (1966)	General structures; stain for acidic and basic components of the tissue
Azure A and eosin B pH 3.7–4.3	10% neutral formalin	Conn *et al* (1960)	General structures
Mallory's phloxine methylene blue	10% neutral formalin	Mallory (1938)	Stain for collagen
Masson's Trichromes	10% neutral formalin	Masson (1929)	Stain for collagen and intracellular fibers
Gomori's Trichromes	10% neutral formalin	Gomori (1950)	Stain for collagen and connective tissues
Phosphotungstic acid hematoxylin	10% neutral formalin and Susa	Lillie (1954)	Stain for collagen
Mallory's collagen stain aniline blue and orange G	Zenker's fluid	Mallory (1936)	Stain for collagen

TABLE 2. METHODS EMPLOYED TO DEMONSTRATE CARBOHYDRATE COMPONENTS AND METACHROMASIA OF THE BARNACLE ADHESIVE(S)

Methods	Fixatives	References	Purposes
Periodic Acid Schiff (PAS)	Susa and 10% neutral formalin	Thompson (1966)	Demonstration of adjacent glycol or amino-hydroxy groupings
PAS + Acetylation	10% neutral formalin	Lillie (1954)	Blocking 1,2-glycols and 1,2-aminohydroxy groups in oxidative Schiff reaction
PAS + Saponification	10% neutral formalin	Lillie (1954)	Deacetylation
PAS + Saliva digestion	10% neutral formalin	Lillie (1949)	Removal of glycogen and RNA
Mucicarmine	10% neutral formalin and Carnoy's fluid	AFIP (1957)	Demonstration of acid mucopolysaccharide (mucin) of epithelial origin
Bast's Carmine	Susa and Carnoy's fluid	Thompson (1966)	Demonstration of glycogen
Azure A O.1% and 0.01% pH 3.9	Susa and Carnoy's fluid	Thompson (1966)	Demonstration of metachromasia
Methenamine silver nitrate	10% neutral formalin	Gomori (1946)	Demonstration of glycogen and mucin
Alcian blue pH 2.8	Susa and Bouin's fluid	Thompson (1966)	Demonstration of sulfated acid mucopolysaccharide
Ribonuclease + Alcian blue pH 2.8	Susa and Bouin's fluid	Thompson (1966)	Hydrolysis of RNA
Alcian blue pH 2.8 + sulfation	Susa and Bouin's fluid	Thompson (1966)	Demonstration of metachromasia by esterification of carbohydrates
Toluidine blue 0, at 0.01% pH 2.8 + sulfation	Susa and Bouin's fluid	Thompson (1966)	Demonstration of metachromasia by esterification of carbohydrates

TABLE 3. METHODS EMPLOYED TO DEMONSTRATE NUCLEIC ACIDS IN THE BARNACLE ADHESIVE(S)

Methods	Fixatives	References	Purposes
Methylene blue pH 3.0	Susa's fluid	Stenram (1953)	Demonstration of nucleic acids; RNA and DNA
Ribonuclease + Methylene pH 3.0	Susa's fluid	Stenram (1953)	Enzymatic hydrolysis of RNA
Toluidine blue 0, 0.5% pH 3.0	Susa's fluid	Stenram (1953)	Demonstration of nucleic acids; RNA and DNA
Ribonuclease + Toluidine blue 0, 0.5%, pH 3.0	Susa's fluid	Stenram (1953)	Enzymatic hydrolysis of RNA
Nucleal Feulgen Reaction	Susa's fluid	Thompson (1966)	Demonstration of DNA

TABLE 4. METHODS EMPLOYED TO DEMONSTRATE LIPIDS AND UNSATURATED FATS IN THE BARNACLE ADHESIVE(S)

Methods	Fixatives	References	Purposes
Sudan black B	Unfixed tissue and 10% neutral formalin	Thompson (1966)	Demonstration of liquid, and semi-solid fats in tissues
Sudan black B + acetone	Unfixed tissue and 10% neutral formalin	Thompson (1966)	As control
Plasmal Reaction	10% neutral formalin	Hayes (1949)	Demonstration of unsaturated fats
Luxol fast blue	10% neutral formalin	Thompson (1966)	Demonstration of phospholipids

TABLE 5. METHODS EMPLOYED TO DEMONSTRATE PROTEIN AND AMINO ACIDS IN THE BARNACLE ADHESIVE(S)

Methods	Fixatives	References	Purposes
Tetrazotized benzidine with β-naphthol	Bouin's Fluid 10% formalin	Lillie (1957)	Demonstration of proteins in general
Ninhydrin Schiff	Susa's fluid	Yasuma *et al* (1953)	Demonstration of the sites of free α-amino acids
2,2′-dihydroxy-6, 6′-dinaphthyl	Susa's fluid 10% formalin	Thompson (1966)	Demonstration of the sulfhydryl group and
disulfide and thioglycolic acid (DDD)	Carnoy's fluid		disulfide linkages
DDD without thioglycolic acid	Susa's fluid	Thompson (1966)	Demonstration of the sulfhydryl group
DDD with benzoyl chloride	Susa's fluid 10% formalin	Thompson (1966)	Demonstration of the disulfide linkages
Mercury Orange	Susa's fluid Carnoy's fluid	Thompson (1966)	Demonstration of the sulfhydryl group
8-hydroxyquinoline	Susa's fluid	Lillie (1957)	Demonstration of amino acid arginine
p-dimethylaminobenz-aldehyde nitrite	Susa's fluid 10% formalin	Adams (1957)	Demonstration of indole derivative (tryptophan)
Diazotization with 8-amino-1-naphthol-5-sulfonic acid	10% formalin	Glenner and Lillie (1959)	Demonstration of tyrosine and phenolic compounds
Millon's Reagent	10% formalin	Thompson (1966)	Localization of tyrosyl groups in tissue sections

Description of the Cement Apparatus[1]

The Cyprid Cement Gland

The cement glands in the cyprid of *Balanus eburneous,* as in the other species of the Balanidae, are paired kidney-shaped structures with an average measurement of $70 \times 50 \times 70$ microns. Each gland consists of a number of secretory cells arranged as compartments. Each secretory cell (Figure 1) contains a large amount of two histochemically distinct secretory granules and a peripherally located nucleus. These granules referred here as medial and inner granules according to their relative position within the cement gland of the cyprid. The α- and β-cells reported by Walker (1971) are not easily discerned with the light microscope. However, the medial and the inner granules are probably the two types of electron-dense bodies found in the α-cells. The collecting canal arises within the medullary portion of the gland and then passes into the conducting canal through the antennule to the attachment pad (Figure 2).

The Adult Cement Apparatus

The cement secreting cells in the adult barnacle are not enclosed in a compact glandular structure; instead the cells are scattered in the mantle tissue mainly along the lateral axis with the associated canals. The cement cells are large, up to 140 microns in diameter, with unusually large polymorphic nuclei containing clumps of chromatin material. The cytoplasm has evenly distributed granules and densely staining secretory zones. No vacuoles were noted at the secretory zones (Figure 3). The smallest cement cell with secretary zones measures 14 microns in diameter; the circular nucleus contains a centrally located nucleolus. The collecting canal makes its contact with the cement cell at secretory zone (Figure 4), and the conducting canal links the individual cement cells into grape-like clusters. The intracellular cement substance accumulated at the secretory zones is delivered to the exterior through the canal system in the basal plate.

The Basal Plate Structures

The basal plate of *Balanus eburneus* Gould is a complicated structure. At the center of the basal plate, the region of the initial point of attachment of the cyprid, the hardened cement consists of two spots approximately 18 microns in diameter, with the remains of the segment IV and the attachment pads of the antennules attached to the substrate. The initial cement of the adult barnacle, located anteriorly to the cyprid cement, also appears as two spots, approximately 35 microns in diameter, with the broken portion of the conducting canal remaining at the center of each spot (Figure 5). Two radial canals are formed by branching along the lateral axis from the conducting canals at the center of the base with circular canals growing out from the radial canals. The adhesive substance is delivered to the edge of the growing plate through the openings in the circular canals. Figure 6 is a composite schematic representation of the cement apparatus of the pre- and post-metamorphosed cyprid at the region of initial attachment.

Histological and Histochemical Reactions

The results of the histological and histochemical reactions on various tissue and cellular components of the cement apparatus of the pre- and post-metamorphosed stages of the barnacle, *Balanus ebureus* Gould, are summarized in Tables 6 to 10.

The medial and the inner granules of the cyprid cement secreting cells show differences in (1) degree of acidophilia; (2) permeability to orange G and hematin-lake formation; and (3) intensity in reaction with carbohydrate and amino acid detecting agents.

The granules in both locations have similar histochemical reactions and stained positively with a number of collagen identifying agents. The medial and the inner granules are characterized by the presence of (1) tryptophan, arginine, and a number of α-amino acids; (2) small amount of lipid and phospholipid substance; (3) no unsaturated fats; (4) carbohydrate components of small molecular size; and (5) sulfhydryl group and disulfide linkage. These two types of granules probably represent stages in synthesis.

The cement material at the secretory zones of the adult cement cell does not differentiate into two acidophilic zones. This fluid cement is also identified as a collagenous substance. The carbohydrate reactions are less intense in comparing with the cyprid inner granules. A small amount of lipid and phospholipid substances are present. Neither unsaturated fats nor ribonucleic acid are found in the cement. No metachromasia is demonstrated. Tyrosine is found in the adult cement but absent in the cyprid of *Balanus eburneus.*

The hardened cyprid cement, i.e. the cyprid antennular deposits at the center of the basal plate, is not stained by the acid and basic dyes and is nearly histologically and histochemically inert. However, the free adjacent glycol or amino-hydroxy groupings can still be detected with PAS. Furthermore, the cement in the hardened state is apparently a protein mass but no specific amino acids could be demonstrated when treated by the usual chemical agents. This observation is in agreement with the histochemi-

[1] A glossary of terms used in this text is given in the appendix.

cal analyses of the cyprid hardened cement by Hillman and Nace (1970).

The hardened adult barnacle cement appears as rings associated with the circular canals on the basal plate, which becomes histochemically non-reactive but has the characteristic of a basic protein. It differs from the hardened cyprid cement by the following characteristics: (1) positive alkaline nature; (2) PAS negative; and (3) the presence of tyrosyl groups.

FIGURE 1. A section through the cyprid cement gland showing the cortically located secretory cells and the collecting canal at the medullary portion of the gland; the cytoplasmic granules are colorless with the tribastic stains. Col C, collecting canal; Se Ce, secretory cells; Ep, epithelial cells. (43 X).

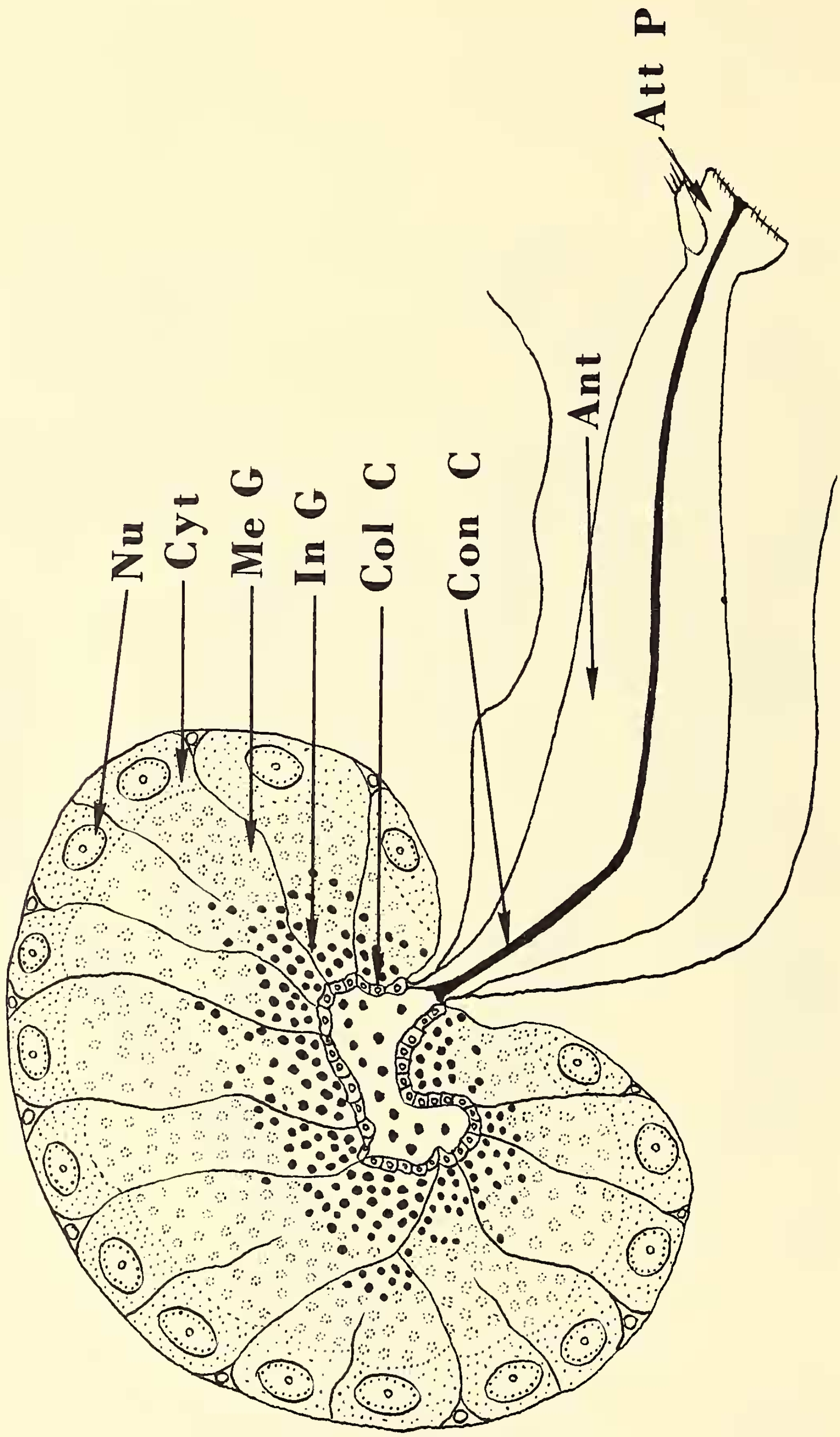

FIGURE 2. A composite schematic representation of the cyprid cement apparatus showing the relative position of the medial and the inner granules within the gland; Nu, nucleus of the secretory cell; Cyt, cytoplasm; Me G, medial granules; In G, inner granules; Col C, collecting canal; Con C, conducting canal; Ant, antennule; Att P, attachment pad.

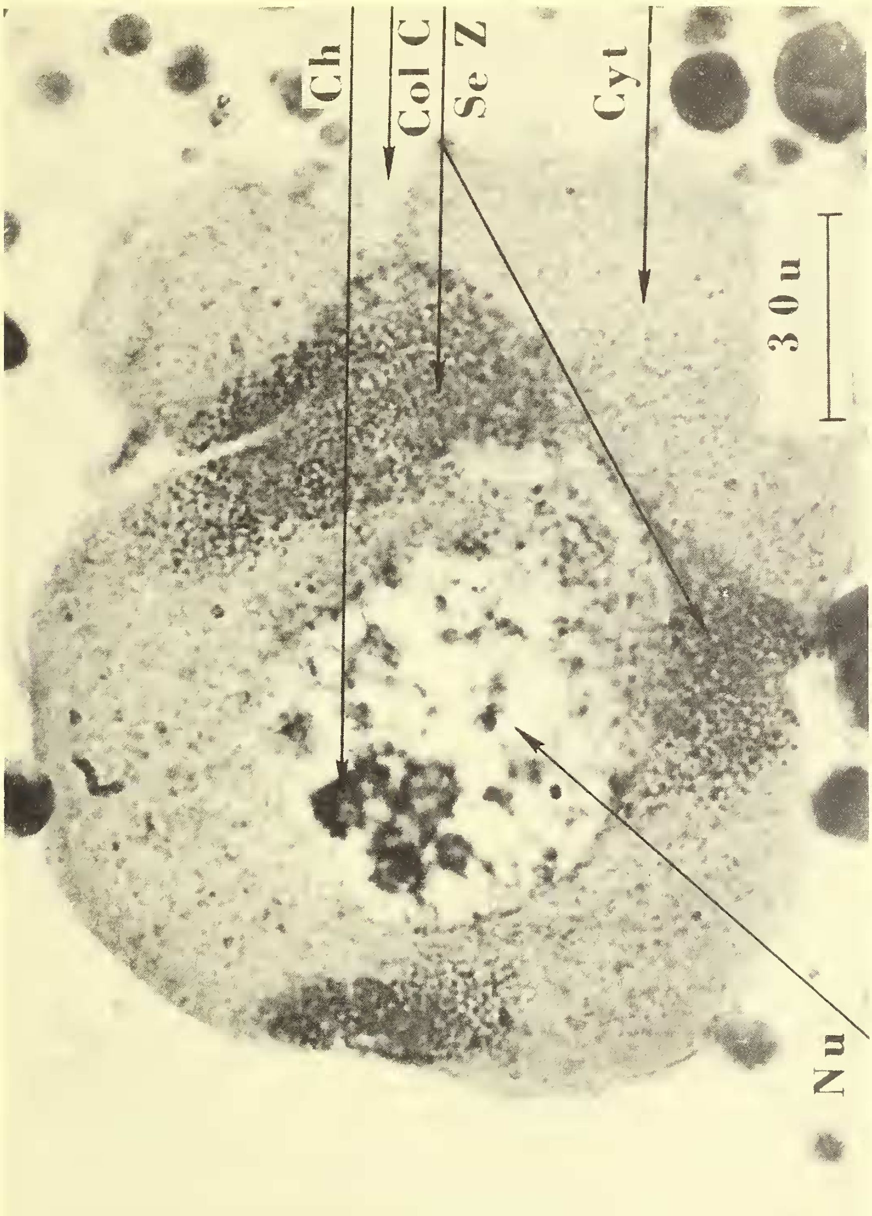

FIGURE 3. A section through an adult cement cell of *Balanus eburneus* Gould; the cytoplasm is grayish and the three secretory zones are pink with Mallory's phloxine stains; Ch, chromatin materials; Col C, collecting canal; Se Z, secretory zone; Cyt, cytoplasm; Nu, nucleus. (100 X).

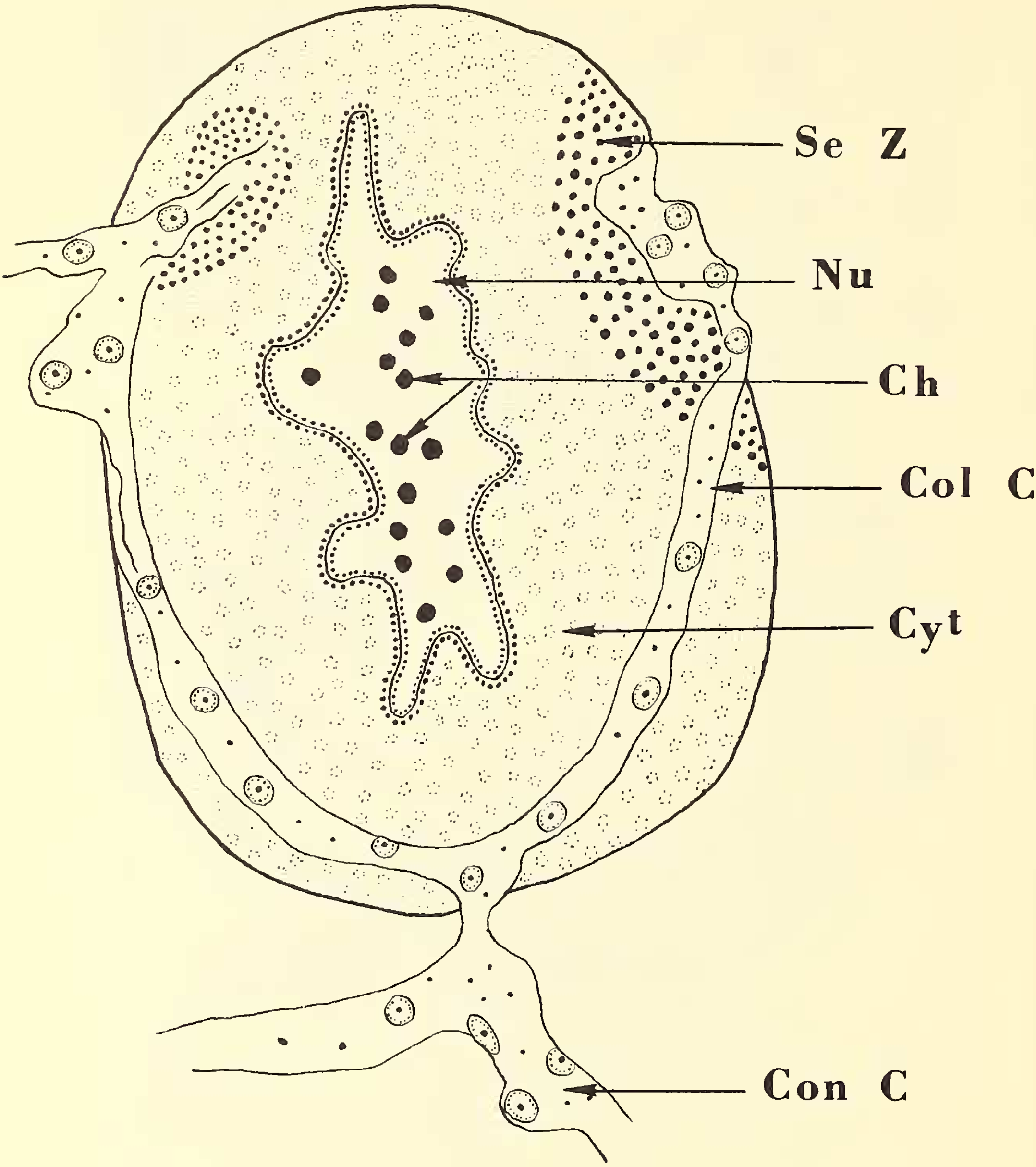

FIGURE 4. A schematic representation of the adult barnacle cement cell associated with the collecting and the conducting canals; Se Z, secretory zone; Nu, polymorphic nucleus; Ch, chromatin materials; Col C, collecting canal; Cyt, cytoplasm; Con C, conducting canal.

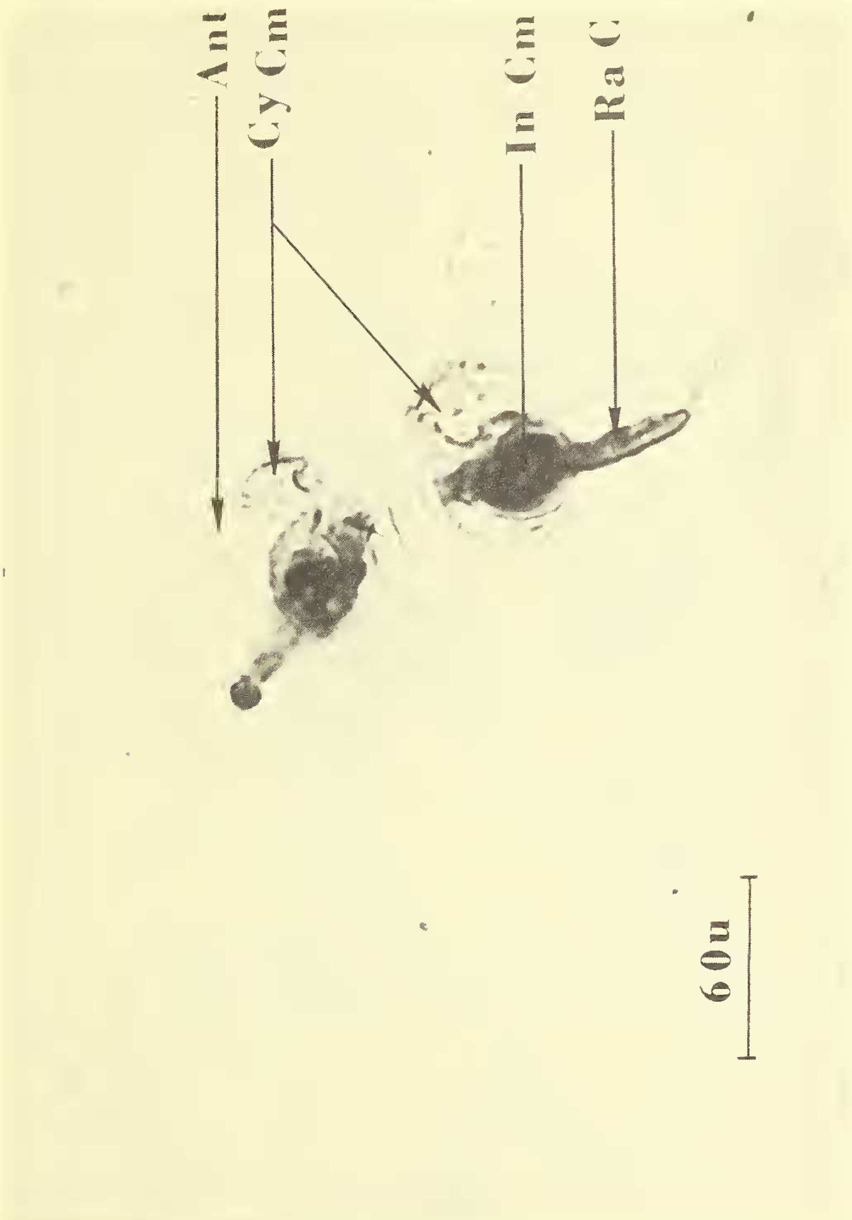

FIGURE 5. The area of initial attachment on the basal plate of *Balanus eburneus* Gould; the cyprid cement is colorless while the adult barnacle cement and the radial canal are pink when treated with Millon's Reagent; Ant, segment IV of the antennule; Cy Cm, cyprid ceme ntwith the antennular attachment pad; In Cm, initial cement of an adult barnacle; Ra C, radial canals branching from the center of the basal plate. (43 X).

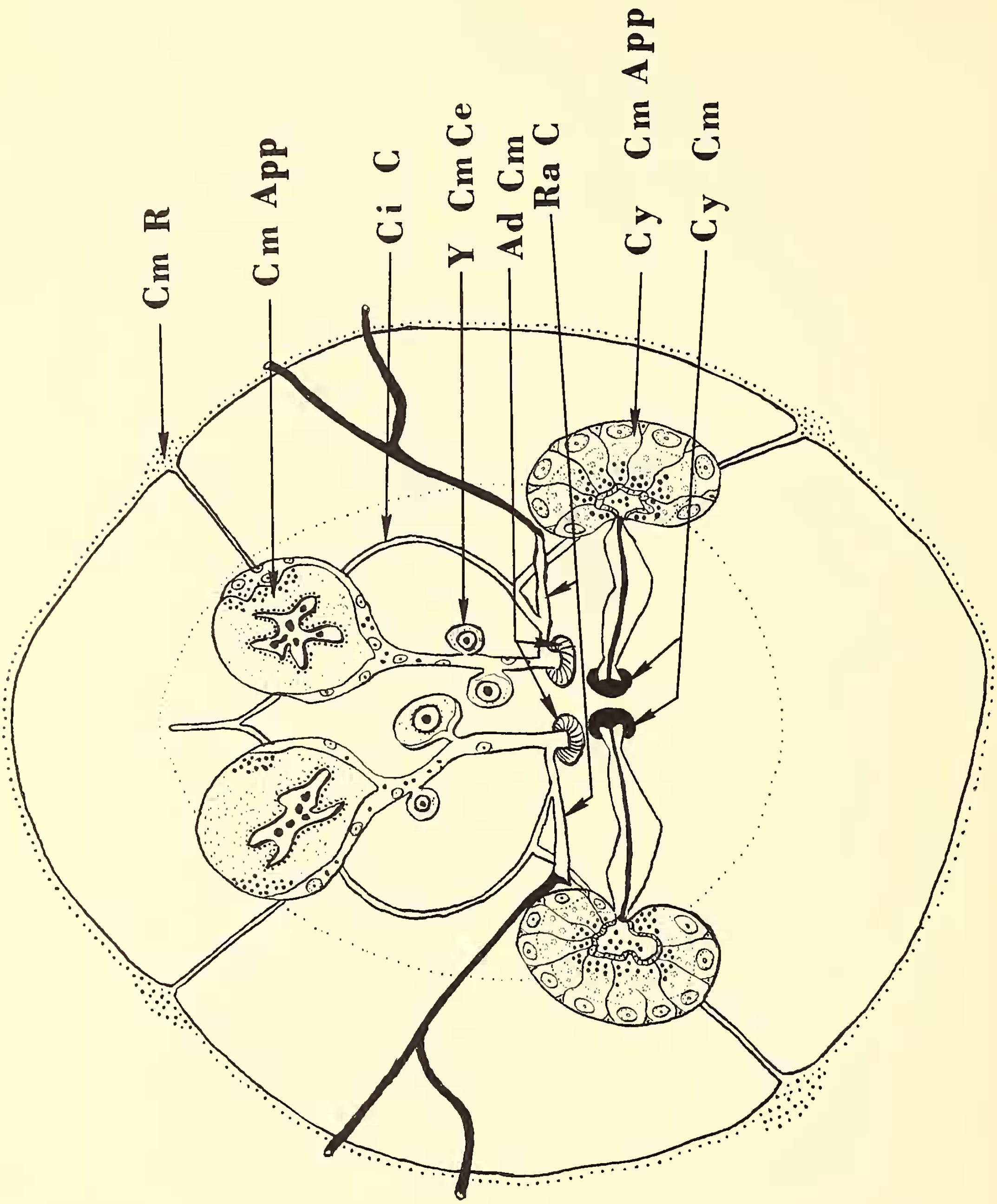

FIGURE 6. A composite schematic representation of the area of the initial attachment made by the barnacle showing the relationship between the cyprid cement gland and the adult cement apparatus; Cm R, cement ring; Cm App, cement apparatus; Ci C, circular canal; Y Cm Ce, young cement cell; Ad Cm, adult cement; Ra C, radial canal; Cy Cm App, cyprid cement apparatus; Cy Cm, cyprid cement.

TABLE 6. GENERAL HISTOLOGICAL AND HISTOCHEMICAL REACTIONS OF VARIOUS COMPONENTS OF THE CEMENT APPARATUS OF THE BARNACLE, *Balanus eburneus* GOULD

Methods	Structures							
	Adult Cement Cell		Cyprid Cement Cell			Basal Plate		
	Cytoplasm	Secretory Zone	Cytoplasm	Medial Granules	Inner Granules	Antennule Deposit	Fluid Cement	Hardened Cement
Hematoxylin and eosin	L. blue	pink	blue	purple	pink	pink	pink	pink
Azure A and eosin B	blue	pink	blue	pink	red	colorless	pink	pink
Mallory's phloxine and methylene blue	blue	red	blue	purple	red	colorless	red	red
Masson's Trichromes	violet	violet	red	blue	red	colorless	red	red
Gomori's Trichromes	violet	violet	red	L. blue	red	colorless	red	red
Phosphotungstic acid Hematoxylin	purple	L. brown	purple	L. brown	blue	colorless	purple	colorless
Mallory's collagen stain aniline blue and orange G	purple	orange	purple	blue	orange	colorless	orange	colorless

TABLE 7. HISTOCHEMICAL TESTS FOR CARBOHYDRATES IN THE BARNACLE ADHESIVES(S)

Methods	Structures							
	Adult Cement Cell		Cyprid Cement Cell			Basal Plate		
	Cytoplasm	Secretory Zone	Cytoplasm	Medial Granules	Inner Granules	Antennule Deposit	Fluid Cement	Hardened Cement
Periodic Acid Schiff (PAS)	L. pink	pink	pink	pink	pink	pink	pink	colorless
PAS + acetylation	colorless	colorless	colorless	colorless	colorless	colorless	colorless	colorless
PAS + Saponification	L. pink	pink	pink	L. pink	L. pink	pink	pink	colorless
PAS + Saliva digestion	colorless	colorless	colorless	colorless	colorless	colorless	colorless	colorless
Mucicarmine	red	red	red	pink	red	colorless	colorless	colorless
Best's Carmine	pink	L. pink	red	pink	pink	......	......	......
Azure A 0.1% and 0.01% at pH 3.9	blue	colorles	bule	colorless	colorless	colorless	colorless	colorless
Methenamine silver nitrate	L. black	black	colorless	L. black	black	colorless	colorless	colorless
Alcian blue pH 2.8	blue	blue	blue	blue	blue	colorless	colorless	colorless
Ribonuclease + Alcian blue pH 2.8	blue	blue	blue	blue	blue	colorless	colorless	colorless
Alcian blue + Sulfation	dissolved	dissolved	blue	blue	blue	L. blue	colorless	colorless
Toluidine blue 0 + Sulfation	dissolved	dissolved	blue	blue	blue	blue	colorless	colorless

TABLE 8. HISTOCHEMICAL TESTS FOR NUCLEIC ACIDS IN THE BARNACLE ADHESIVE(S)

Methods	Structures							
	Adult Cement Cell		Cyprid Cement Cell				Basal Plate	
	Cytoplasm	Secretory Zone	Cytoplasm	Medial Granules	Inner Granules	Antennule Deposit	Fluid Cement	Hardened Cement
Methylene blue	blue	blue	blue	colorless	colorless	colorless	colorless	colorless
Ribonuclease + Methylene blue	colorless	colorless	colorless	colorless	colorless	colorless	colorless	colorless
Toluidine blue 0	blue	colorless	blue	colorless	colorless	colorless	colorless	colorless
Ribonuclease + Toluidine blue 0	colorless	colorless	colorless	colorless	colorless	colorless	colorless	colorless
Feulgen Reaction	colorless	colorless	colorless	colorless	colorless	......	......	......

TABLE 9. HISTOCHEMICAL TESTS FOR LIPIDS AND UNSATURATED FATS IN THE BARNACLE ADHESIVE(S)

Methods	Structures							
	Adult Cement Cell		Cyprid Cement Cell				Basal Plate	
	Cytoplasm	Secretory Zone	Cytoplasm	Medial Granules	Inner Granules	Antennule Deposit	Fluid Cement	Hardened Cement
Sudan black B	colorless	blue	L. black	L. black	colorless	colorless	L. black	colorless
Sudan black B + Acetone	colorless	colorless	colorless	colorless	colorless	colorless	colorless	colorless
Plasmal Reaction	L. pink	colorless	L. pink	colorless	colorless	colorless	colorless	colorless
Luxol fast blue	colorless	blue	colorless	colorless	blue	blue	blue	blue

TABLE 10. HISTOCHEMICAL TESTS FOR PROTEINS AND AMINO ACIDS IN THE BARNACLE ADHESIVE(S)

Methods	Structures							
	Adult Cement Cell		Cyprid Cement Cell			Basal Plate		
	Cytoplasm	Secretory Zone	Cytoplasm	Medial Granules	Inner Granules	Antennule Deposit	Fluid Cement	Hardened Cement
Tetrazotized benzidine with β-naphthol	orange	orange	orange	orange	orange	orange	orange	orange
Ninhydrin Schiff α-amino acids	pink	pink	pink	L. pink	pink	pink	pink	pink
2,2′-dihydroxy-6,6′-dinaphthyl disulfide (DDD) for SH- & S-S	brownish	purple	orange	L. orange	purple	colorless	L. purple	colorless
DDD without thioglycolic acid for SH- group	orange	brownish	orange	orange	orange	colorless	L. purple	colorless
DDD with benzoyl chloride for S-S	brownish	purple	orange	orange	purple	colorless	L. purple	colorless
Mercury Orange	orange	L. orange	orange	L. orange	orange	colorless	orange	colorless
8-hydroxyquinoline for arginine	orange	orange	orange	orange	orange	colorless	orange	colorless
p-dimethylamino-benzaldehyde nitrite for tryptophan	colorless	blue	colorless	blue	blue	colorless	blue	colorless
Diazotization with 8-amino-1-naphthol-5-sulfonic acid for tyrosine	L. orange	orange	colorless	colorless	colorless	colorless	violet	violet
Millon's Reagent for tyrosine	L. orange	orange	colorless	colorless	colorless	colorless	red	orange

Discussion

Histological observations on serial sections of a newly metamorphosed barnacle show that the adult cement apparatus is not derived from the remnant of the cyprid cement gland. Therefore, these two cement producing organs have different origins and developments. Based on the morphological differences of the cement apparatus, it is assumed that the adhesive substances produced by the pre- and post-metamorphosed cyprid are different.

Therefore, it should be emphasized that our histochemical studies deal with the following: (1) the intracellular fluid material of the cyprid cement gland; (2) the "hardened" cement at the points of antennular attachment of the cyprid; (3) the intracellular and intracanicular fluid cement of the post-metamorphosed barnacle; and (4) the "hardened" cement of the initial and subsequent deposits produced by the adult.

Further, it should be recognized that our studies deal mainly with the surface chemistry of the so-called "hardened" cement of the cyprid and adult, which may account for some of the differences in the histochemical reactions noted by us and those reported by other investigators (Walker 1970, 1971; Hillman and Nace, 1970; and Saroyan, *et al,* 1970a).

The histochemical analyses of the fluid and the solid state of the cement suggest that the materials are collagenous which undergo a transition from a highly chemically reactive (fluid cement) to an unreactive mass upon hardening.

The fluid cement of the cyprid is present in the treated material as medial and inner granules characterized histochemically by differences in the numbers of free cationic groups as reflected by differences in the degree of acidophilia of these two granules. It is our belief that these granules are the same as those reported by Walker (1971) as two types of electron-dense bodies within the α-cells of the cyprid cement gland. It further leads us to infer that the inner granules (i.e., more electron-dense bodies) are probably a polymerized state of the medial granules (i.e., less electron-dense bodies), which may represent the monomeric state of the adhesive. The above interpretations may also explain the difference in the intensity of most of the histochemical reactions of these two types of granules.

All of the histochemical tests, as mentioned above, indicate that the fluid cement of both the pre- and post-metamorphosed cyprids is a collagenous substance. The presence of sulfhydryl groups and disulfide linkage indicates that the structure of the cement has a compactly coiled and/or folded configuration.

The presence or absence of tyrosine in the cement material raises some questions. Our results showed that this amino acid is not present in the fluid and hardened cement of the cyprid (see also Hillman and Nace, 1970), but is found in the cement of the adult (Figure 5). This is contrary to that reported by Saroyan *et al* (1970a) and by Walker (1970, 1971) who demonstrated tyrosine in the cement of both the adult and cyprid. The significance of the lack of the demonstrable tyrosyl group in the cyprid cement is obscure.

The interpretation that the cement is an acid mucopolysaccharide, as has been suggested by several investigators, can be ruled out by the absence of metachromasia with or without prior sulfation indicating that the carbohydrate component is of small molecular size with few anionic and hydroxyl groups.

The fluid cement undergoes considerable changes during the hardening process which, in our observations, appears to take place at the site of cement-substratum junction. The mechanism(s) of hardening has not been firmly established. There are some indications, however, that cement hardening agents and/or enzymes are present in the mantle tissue (Shimony and Nigrelli, 1971b).

Conclusion and Summary

On the basis of the selective histochemical tests employed in these studies, it is concluded that the fluid cement (intracellular and intracanicular) in the cyprid and adult stages of the barnacles are collagenous substances. The fluid cement produced by the gland cells of the adult barnacle differs from that formed in the cyprid by the presence of tyrosine, and by a less intense color reaction for carbohydrates. The significance of these differences remains obscure at this time.

The antennular deposits of the cyprid and cement particles in the basal plate produced by the adult barnacle are referred to as solid or hardened cement. The hardened cyprid cement is PAS-positive but non-reactive to acidic and basic dyes; the hardened adult cement is PAS-negative and is stained readily with acidic dyes and, as would be expected, gives a positive reaction for the presence of tyrosyl groups.

Appendix

1. Medial Granules: stainable materials found proximal to the nucleus of the secretory cells of the cyprid cement gland.
2. Inner Granules: acidophilic granules found distal to the nucleus of the secretory cells of the cyprid cement gland.
3. Secretory Zones: the acidophilic zones in the adult cement cells where the fluid cement is accumulated for secretion.

4. Collecting Canal: the canal that penetrates the medullary portion of the cyprid cement gland or the canal that is associated with the adult cement cells at the secretory zones.
5. Conducting Canal: the canal that passes within the cyprid antennule and terminates at the attachment pad; in the young barnacle, it is the canal that links the collecting canals of individual cement cells and runs perpendicular to the radial and circular canals.
6. Radial Canals: the two largest canals on the basal plate extending radially along the lateral axis from the center of the basal plate where the adult cement initiates.
7. Circular Canal: the canal that branches out of the radial canal and forms rings on the basal plate. It delivers cement to the exterior through the orifices along the canals.
8. Cyprid Antennular Deposits: the hardened cement which is deposited by the cyprid at the initial point of attachment.

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